

clinical signs noted and recorded each day.

(iv) If three of three controls do not show clinical signs of feline rhinotracheitis virus infection other than fever, the test is inconclusive and may be repeated.

(v) If a significant difference in clinical signs cannot be demonstrated between vaccinates and controls using a scoring system approved by Animal and Plant Health Inspection Service and prescribed in the Outline of Production, the serial is unsatisfactory.

[50 FR 433, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

**§ 113.212 Bursal Disease Vaccine, Killed Virus.**

Bursal Disease Vaccine, Killed Virus, shall be prepared from virus-bearing cell culture fluids or embryonated chicken eggs. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable requirements prescribed in § 113.200.

(b) Each lot of Master Seed shall be tested for pathogens by the chicken embryo inoculation test prescribed in § 113.37, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in § 113.36 may be conducted and the virus judged accordingly.

(c) The immunogenicity of vaccine prepared in accordance with the Outline of Production shall be established by a method acceptable to Animal and Plant Health Inspection Service. Vaccine used for this test shall be at the highest passage from the Master Seed and prepared at the minimum preinactivation titer specified in the Outline of Production. The test shall establish that the vaccine, when used as recommended on the label, is capable of inducing an immune response in dams of sufficient magnitude to provide significant protection to offspring.

(d) *Test requirements for release.* Each serial and subserial shall meet the ap-

plicable general requirements prescribed in § 113.200 and the special requirements in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety.* Vaccinates used in the potency test in paragraph (d)(2) of this section shall be observed each day during the prechallenge period. If unfavorable reactions attributable to the vaccine occur, the serial is unsatisfactory. If unfavorable reactions which are not attributable to the vaccine occur, the test is inconclusive and may be repeated. If the test is not repeated, the serial is unsatisfactory.

(2) *Potency.* Bulk or final container samples of completed product from each serial shall be tested for potency using the two-stage potency test provided in this paragraph.

(i) *Vaccinates.* Inject each of 21 susceptible chickens 14 to 28 days of age, properly identified and obtained from the same source and hatch, with one dose of vaccine by the route recommended on the label and observe for at least 21 days.

(ii) *Controls.* Retain at least 10 additional chickens from the same source and hatch as unvaccinated controls.

(iii) *Challenge.* Twenty-one to 28 days postvaccination, challenge 20 vaccinates and 10 controls by eyedrop with a virulent infectious bursal disease virus furnished or approved by Animal and Plant Health Inspection Service.

(iv) *Postchallenge period.* Four days postchallenge, necropsy all chickens and examine each for gross lesions of bursal disease. For purposes of this test, gross lesions shall include peribursal edema and/or edema and/or macroscopic hemorrhage in the bursal tissue. Vaccinated chickens showing gross lesions shall be counted as failures. If at least 80 percent of the controls do not have gross lesions of bursal disease in a stage of the test, that stage is considered inconclusive and may be repeated. In a valid test, the results shall be evaluated according to the following table:

Stage	Number of vaccinates	Cumulative number of vaccinates	Cumulative total number of failures for—	
			Satisfactory serial	Unsatisfactory serial
1 .....	20	20	3 or less .....	6 or more.
2 .....	20	40	8 or less .....	9 or more.

(v) If four or five vaccinates show lesions of bursal disease in the first stage, the second stage may be conducted in a manner identical to the first stage. If the second stage is not conducted, the serial is unsatisfactory.

(vi) If the second stage is used, each serial shall be evaluated according to the second part of the table on the basis of cumulative results.

[50 FR 434, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

#### § 113.213 Pseudorabies Vaccine, Killed Virus.

Pseudorabies Vaccine, Killed Virus, shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.200.

(b) The immunogenicity of vaccine prepared from the Master Seed in accordance with the Outline of Production shall be established by a method acceptable to Animal and Plant Health Inspection Service. Vaccine used for this test shall be at the highest passage from the Master Seed and at the minimum preinactivation titer provided in the Outline of Production.

(c) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in § 113.200 and the special requirements provided in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety.* Vaccinates used in the potency test in paragraph (c)(2) of this section shall be observed each day during the prechallenge period. If unfavorable reactions occur, including neuro-

logical signs, which are attributable to the vaccine, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the vaccine, the test is inconclusive and may be repeated. If the test is not repeated, the serial is unsatisfactory.

(2) *Potency.* Bulk or final container samples of completed product shall be tested for potency as follows:

(i) Ten pseudorabies susceptible pigs (five vaccinates and five controls) shall be used as test animals. The animals shall be at the minimal age recommended for vaccination. Blood samples shall be drawn and individual serum samples inactivated and tested for neutralizing antibody.

(ii) A constant virus-varying serum neutralization test in cell culture using 50 to 300 TCID<sub>50</sub> of virus shall be used. Pigs shall be considered susceptible if there is no neutralization at 1:2 final serum dilution. Other tests of equal sensitivity acceptable to Animal and Plant Health Inspection Service may be used.

(iii) The five pigs used as vaccinates shall be administered one dose of vaccine as recommended on the label. If two doses are recommended, the second dose shall be given after the interval recommended on the label.

(iv) Fourteen days or more after vaccination, blood samples shall be drawn and individual serum samples inactivated and tested for pseudorabies virus neutralizing antibody by the method used to determine susceptibility.

(v) *Test interpretation.* If the controls have not remained seronegative at 1:2, the test is inconclusive and may be repeated. If at least four of the five vaccinates in a valid test have not developed titers of at least 1:8, and the remaining vaccinee has not developed a titer of at least 1:4, the serial is unsatisfactory, except as provided in paragraph (c)(2)(vi) of this section.